Nonrheumatic calcific aortic stenosis: an overview from basic science to pharmacological prevention

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Summary

Calcific aortic stenosis is a frequent degenerative disease, which represents the most common indication for adult heart valve surgery, and carries substantial morbidity and mortality. Due to ageing populations in western countries, its prevalence is expected to increase in the coming years. Basic science studies suggest that the progression of aortic valve stenosis involves an active biological process, and that the molecular mechanisms promoting this development resemble those of atherosclerosis, as stenotic aortic valves are characterized by complex histological lesions, consisting of activated inflammatory cells, lipid deposits, extracellular matrix remodeling, calcific nodules, and bone tissue. This has led to the hypothesis that drugs effective in delaying atherosclerosis progression (e.g. statins) might also be able to prevent the progression of calcific aortic valve stenosis. The potential benefit of statin therapy, however, is controversial and widely debated, as recent randomized studies done in patients with moderate to severe degrees of aortic stenosis failed to consistently show substantial benefits of this class of drugs. This review focuses on various aspects of molecular mechanisms underlying calcific aortic valve stenosis and discusses recent experimental and clinical studies that address the potential benefit of targeted drug therapies. Taken together, current evidence suggests that the progression of calcific aortic stenosis is a multi-factorial process; the multitude of the mechanisms potentially involved in aortic valve stenosis indicates that drug therapy aimed at reducing its progression is necessarily multi-factorial and should address the earliest stages of the disease, as it is now evident that pharmacological treatment administered in more advanced stages of the disease may be ineffective or, at best, much less effective.

Keywords: Calcific aortic valve stenosis; Atherosclerosis; Statins

1. Introduction

Calcific aortic valve stenosis is the most common heart valve disease in the western world, especially in elderly people [1,2]; on average, 50,000 aortic valve replacements every year occur both in Europe and in the United States due to this pathology, which is the most common valvular disease of the adult [3,4]. The prevalence of clinically significant aortic stenosis increases progressively with age: it is around 2% in people over 65 [5], and it is more than 4% in octogenarians [6]. The behavior of aortic valve sclerosis, a milder form of aortic valve disease characterized by calcification and stiffening of the aortic valve without a transvalvular gradient, parallels the one of calcific stenosis, the prevalence being around 20–30% in patients aged over 65 years [5,6], and reaching 48–57% in octogenarians [6,7].

This translates in very high costs for health organizations that are estimated to be around 1 billion US dollars per year in the United States [1], consequently leading to great interest in medical therapies that could potentially slow the progression of this disease.

In recent years, the assumption that calcific aortic stenosis is a passive, age-related disease has been strongly questioned by studies showing several similarities and some dissimilarities to atherosclerosis in this evolving pathological process and consequently studies investigating the potential role of the most diffuse anti-atherosclerotic drugs, the statins, have been carried out with, unfortunately, controversial results.

In this paper we review the current knowledge on molecular bases of aortic sclerosis and stenosis, the potential preventive therapies, and the results coming from recent clinical trials.
2. Genomics

Several genomic studies have assessed the possible association between calcific aortic stenosis and genetic factors, concerning mainly atherosclerosis and bone metabolism. Regarding atherosclerosis, even if earlier studies have documented a possible link between lipid metabolism and progression of aortic valve disease [8], studies evaluating the prevalence of apolipoprotein E [9–11], Al [9], e B [9] in patients with aortic stenosis have shown conflicting results. In patients with calcific aortic stenosis there is a prevalence of the allele X+/X+ of apolipoprotein B, but similar allelic frequencies of A alleles [9], whereas it is actually unclear whether apoE allelic variants differ in patients with aortic stenosis from controls; some studies have documented a higher prevalence of apoE2 [9] and of apoE4 [11] in aortic stenosis, but these data have not been confirmed by others [10]. Overall, the question whether the frequency of the allelic variants of these lipoproteins is different in aortic stenosis with respect to general population still remains unanswered.

The role of inflammation has also been deeply investigated. Some polymorphisms of the interleukin-10 gene promoter, namely –1082, –819, –592, are associated with the extent of calcium content of stenotic aortic valves excised during surgical intervention, and the effect of these alleles is further potentiated in patients simultaneously carrying the rare chemokine receptor 5 and connective tissue growth factor alleles [12].

Bone metabolism genomics has been investigated through the assessment of vitamin D receptor genetic polymorphism (BsmI B/b) [13], that predicts bone density or bone mineral mass [14]; patients with aortic valve stenosis show higher frequencies of the B allele, which is associated with reduced calcium absorption, to more rapid bone loss with advancing age, and to higher parathormone levels [13], suggesting that the bone metabolism profile favoring calcium mobilization from bone could promote aortic valve calcification. In addition, it has been recently shown that a nonsense mutation of the NOTCH1 gene is associated with early developmental defects and to late de-repression of calcium deposition causing aortic valve disease progression [15]. It is known that NOTCH1 gene plays an important role in hematopoiesis, in stem cell signaling and in cell differentiation during organogenesis, it can also act as a repressor of the transcriptional activity of Runx2, which is important for osteoblast activity, and its inhibition may cause a de-repression of calcium deposition in the aortic valve [15].

Preliminary evidence also suggests a possible role in aortic valve calcification process for Plvul1 polymorphism of the alpha estrogen receptor, possibly linked to the prevalence of aortic valve calcification in post-menopausal women [16], likely through an increase of cholesterol [16].

Finally, some cell cycle regulatory genes may contribute to calcific aortic disease progression, as it has been shown that the expression of p21, WAF1/CIP1 (cyclin-dependent protein-kinase inhibitor p21), and of 14-3-3 σ is reduced, both at a transcriptional and translational level, in calcific aortic valves [17]. This points to cell cycle control as an attractive potential target for future therapies aimed at reducing the progression of aortic valve disease, although evidence to date suggests that only bone metabolism polymorphisms have a definite role in the progression of aortic valve disease and the role of lipid metabolism genomics and of cell cycle regulation is still at the hypothesis level.

3. Atherosclerosis

The development and progression of calcific aortic stenosis is an active atheroinflammatory process consisting of local inflammatory components and deposition of plasma lipoproteins in the lesions; this process shares several features with atherosclerosis with, however, some important differences.

Initial lesions of aortic valve disease are quite similar to atherosclerosis by nature and include disruption of the basement membrane, subendothelial accumulation of intra-cellular lipids and lipoproteins, and infiltration of foam cells, nonfoam cells, and T lymphocytes, together with local and systemic activation of inflammation [2,18]. On the other hand, calcification is more extensive in aortic valve disease than in atherosclerosis, and fibrocalcific thickening is responsible for the clinical manifestations of the disease [19,20]. In this section we resume the studies that have addressed the possible relation between aortic valve disease (sclerosis and stenosis) and atherosclerosis.

3.1. Endothelial function

Patients with aortic valve sclerosis have impaired endothelium-dependent, post-ischemic, flow-mediated dilatation [21]; in addition, aortic valve sclerosis is associated with higher intima-media thickness, a marker of early atherosclerotic vascular structural changes [22], with respect to normal subjects [23]. This further confirms and extends the current concept that relates aortic valve sclerosis, atherosclerosis, and increased risk of future cardiovascular events for patients affected by valve sclerosis [24–27].

Endothelial damage in patients with calcific aortic valve disease is further supported by the demonstration of increased E-selectin plasma levels in patients affected by severe aortic stenosis which return to normal after surgery [28]. The evidence showing higher circulating levels of endothelial microparticles that are correlated with the number of activated monocytes [29], provides another link between aortic disease and atherosclerosis. Finally, studies performed on aortic valve specimens collected at intervention time have shown that diseased aortic valves express more markedly several endothelial markers such as CD31, CD34, von Willebrand factor, and CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule), with respect to normal valves [30], further supporting the association between endothelial dysfunction and calcific aortic valve disease progression.

3.2. Lipid metabolism

Among traditional risk factors for atherosclerosis, lipid metabolism abnormalities have been frequently associated with calcific aortic valve disease. In 1994 Otto et al. described the possible links between lipid metabolism and
calcific aortic disease showing, in diseased aortic valves, the presence of large amounts of intracellular and extracellular neutral lipids that could not be found in normal valves [27]; since then, hyperlipemia has been widely investigated as a possible mechanism underlying aortic valve stenosis development and progression, and several studies support a strong association between lipid metabolism and aortic valve disease. It has been recently shown that plant sterols accumulate in stenotic aortic valves in a direct relation to their respective serum concentrations [31]; this is a proof of concept that lipid metabolism is strictly related to the development and progression of aortic valve disease, as it clearly demonstrates that circulating lipids are capable of entering into aortic valve leaflets and thus to exert local effects in the interstitium of aortic valves. In addition, angiotensin converting enzyme co-localizes with circulating LDL and apolipoprotein B in sclerotic and stenotic aortic valves [32], suggesting that angiotensin converting enzyme may be concentrated in aortic lesions through the retention of plasma lipoproteins. Moreover, stenotic aortic valves contain increased levels of low-density lipoprotein receptor-related protein-5 (LRP5) [33], a member of the low-density lipoprotein receptor-related protein family of cell-surface receptors that are involved in diverse biologic processes, including lipid metabolism, retinoid uptake, and neuronal migration; LRP5 plays an important role in the activation of skeletal bone development and in the differentiation process of aortic valve mesenchymal myofibroblasts into osteoblasts, providing another link between lipid metabolism and aortic valve calcification [33].

Additional evidence suggesting a strong relationship among lipid metabolism, atherosclerosis and aortic valve disease comes from the postmortem analysis of the aortic valve of a 7-year-old boy who died in the 1950s because of type IIb familial hypercholesterolemia; even in this young boy, atherosclerotic lesions with plaques rich in lipid-laden foam cells, focal areas of collagen and scant basophilic round substances were found in aortic valve tissue [34].

From a clinical standpoint, studies have also addressed the potential role of atherosclerosis risk factors in the development and progression of calcific aortic stenosis, showing that high LDL and lipoprotein (a) levels are risk factors both for aortic valve sclerosis [6,35,36] and for stenosis as well [37], even if recent studies limit that effect to patients less than 65 years old [38]. In addition, it has also been shown that metabolic syndrome is associated with aortic stenosis progression over time and this can be explained in great part by the fact that patients affected by metabolic syndrome had greater LDL cholesterol levels than controls (124 mg/dl vs 85 mg/dl) [39]. That hypercholesterolemia is a causative factor for aortic valve disease is not a new concept, as in 1997 Wilmshurst et al. showed that patients suffering from aortic valve stenosis had higher total cholesterol levels [8]; more recently, Pohle et al. have documented that hypercholesterolemic subjects (LDL > 130 mg/dl) have much greater progression of aortic valve calcifications over time, with an average annual increase of calcium of 43% versus an increase of 9% in controls (p < 0.001) [40]. Besides LDL, the overall lipid profile seems to play a role in aortic valve disease, as not only high LDL levels but also high total cholesterol, low HDL levels, and a higher total cholesterol/HDL ratio are independently associated with higher progression rates [41]; also particle size affects progression of aortic valve disease, since small circulating LDL particles (<255 Å) are increased in patients with faster disease progression [42]. This could be related to greater accumulation of oxidized LDL in aortic valve tissue favoring fibrocalcific remodeling of the aortic valve, providing additional arguments that support the hypothesis that aortic stenosis is a lipid-driven process [43]. Finally, Busseuil et al. have shown that, in rabbits, the infusion of an ApoA-1 mimetic peptide leads to the regression of cholesterol-induced aortic valve stenosis, suggesting alternative molecular targets for medical treatment of this disease [44].

3.3. Inflammation

For more than a decade it is well known that aortic valve 'early lesions' are characterized by inflammatory features including T lymphocytes and macrophages [27], but the contribution of inflammation and its mediators has recently become more detailed as described by growing evidence. Several inflammation mediators accumulate in diseased aortic valves: the terminal complement complex C5b-9 is found in sclerotic and even more in stenotic aortic valves [45], that also show increased expression of C3a and C5a receptors [45]; in addition, stenotic aortic valves show increased expression of interleukin-1β and of leukocyte infiltrates [46], of transforming growth factor-β1 (TGF-β1) [47] (and this could promote calcification through apoptosis induction of the interstitial aortic cells [48]), of extracellular matrix tumor necrosis factor-α (TNF-α) [49], and of endothelial adhesion molecules intercellular adhesion molecule-1 (ICAM-1) [28,50,51], of vascular cell adhesion molecule-1 (VCAM-1) [28,51], of Heat Shock Protein-60 [51], of vascular adhesion protein-1, eotaxin-3 and of monokine induced by interferon-gamma [47]; such a variety of inflammation mediators strongly suggests a sensible contribution of inflammation in the course of aortic valve disease.

Of note also is the fact that normal aortic valves have a substantial expression of the Toll-like receptors 2 and 4, which are known to play an important role in pro-inflammatory cytokine expression in several cell populations, especially in interstitial cells [52]; in vitro studies have also shown that Toll-like receptors are fully functional in interstitial aortic valve cells, as peptidoglycan or lipopolysaccharide stimulation can cause NF-κB pathway activation with consequent production and release of pro-inflammatory cytokines, e.g. interleukin-6 — IL-8 — ICAM-1, and of the osteogenesis-related factors bone morphogenetic protein-2 and Runx2 [52], supporting a causative link between inflammation and osteogenesis in aortic valve disease. Such a finding is further supported by evidence concerning the pathway osteoprotegerin/RANK (receptor activator of nuclear factor-κB)/RANKL [53,54] that will be wider discussed in the osteogenesis section.

3.4. Hemostasis

Aortic valve stenosis has for long time been associated with bleeding disorders; in 1958 Heyde described the
association between frequent gastrointestinal bleeding episodes and aortic valve stenosis (Heyde's syndrome) [55]; later, it has been reported that the bleeding in context of aortic stenosis is due to acquired type 2A von Willebrand disease, characterized by a decrease of the large multimers of the von Willebrand factor due to the action of ADAMTS-13, a matrix metalloproteinase that acts on von Willebrand factor preferentially under conditions of high shear stress (e.g. stenotic aortic valves) [56,57]. This phenomenon leads in some cases to clinically significant bleeding episodes [58,59], such as recurrent gastrointestinal bleedings sometimes associated with angiodysplasia [55,58,59]; of note, bleeding episodes can sometimes be controlled only by aortic replacement, when von Willebrand factor multimers return to normal levels [55,58—60]. This perturbation of von Willebrand factor multimers also affects platelet function with a reduction in platelet count [56], prolonged bleeding time [60], impaired shear-induced platelet aggregation [56], prolonged closure time at platelet function analyzer [57], and lower P-selectin levels with respect to controls [61]. Usually, von Willebrand factor levels and multimers return to normal within 6 months after surgery [56,57,59], with the exclusion of patient-prosthesis mismatch [57,58], although not all studies agree upon this. In fact, Yoshida et al., using immunoblotting electrophoresis, have shown a direct linear relation between von Willebrand factor antigen and the indexed effective orifice area of the valve prostheses [59]; on the other hand, Goldsmith et al., using ELISA, have documented an inverse relation between plasma von Willebrand factor and the size of the aortic prosthesis [61]. In summary, evidence concerning von Willebrand factor behavior before and after surgery for aortic valve disease, as well as the differences in the return to baseline in patients with or without patients-prosthesis mismatch suggest that the abnormalities related to this molecule are not causative of aortic valve disease but much more likely its consequences.

Less information is, unfortunately, available about other haemostatic variables; preoperative fibrinogen levels are higher in patient candidates for aortic valve replacement with respect to controls [61], and this could be due to an increased pro-inflammatory status. In addition, patients affected by aortic or mitral disease have a more marked activation of coagulation and fibrinolysis, being prothrombin factor F1.2, thrombin—antithrombin complex and d-dimer higher than in controls, even if it is actually not known whether aortic and mitral valve disease differ somehow in this aspect [62]. Moreover, hypertensive patients who also have aortic valve sclerosis features show higher levels of prothrombin factor F1.2 with respect to hypertensive patients without aortic valve sclerosis [63]. Finally, experimental evidence on animals suggests that an atherogenic diet (cholesterol-rich or cholesterol-rich plus the addition of vitamin D) promotes the development of aortic valve sclerosis together with tissue factor expression on the aortic side of the leaflets [64].

In summary, current knowledge concerning aortic valve disease and coagulation, fibrinolysis, and platelet activation markers suggest also that these pathways can be involved in aortic valve disease progression, but additional evidence is necessary to prove these potential associations.

3.5. Angiogenesis

Angiogenesis, defined as the outgrowth of new vessels from pre-existing blood vessels [65], is regulated by a balance between angiogenic activators such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 and platelet-derived growth factor and inhibitors [66], and is one of the main mechanisms involved in atherosclerosis promoting plaque growth, intraplaque hemorrhage, and lesion instability [67,68]. Several studies have shown that neangiogenesis occurs in the course of calcific aortic disease, and a substantial association with inflammatory infiltrates [30,51,69,70] occurs; interestingly, vascular density is higher in aortic valves with a low or intermediate grade of calcification and lower or even null in severely stenotic valves [69,70], suggesting that neangiogenesis has a specific temporal pattern in the development of this disease. Neovascularization is also associated with increased expression of VEGF and of its receptors Flt-1 and Flk-1, of endothelial nitric oxide synthase [69], and of SPARC (secreted protein, acidic and rich in cysteine/osteonectin) [70], an extracellular matrix protein contributing to embryonic development, blood vessels formation, and tissue remodeling [71]. Further proof concerning angiogenesis role in calcific aortic disease comes also from ex vivo studies showing that new capillary sprouts exhibit endothelial markers as CD31, von Willebrand factor, CD34, and tyrosine kinase receptor Tie-2 on their surface. Finally, Chalajour et al. have identified, in diseased valves, the presence of a cell population expressing both endothelial (VEGF receptor-2 and tyrosine chine receptor Tie-2 expression) and mesenchymal (smooth muscle alpha-actin expression) markers and with an enhanced angiogenic activity that could promote the pathological angiogenesis occurring in stenotic valves via the differentiation of these cells into the cell types needed for this process [72]. Taken together, these data suggest that angiogenesis is one of the players in the field of calcific aortic stenosis, being a potentially new therapeutic target to slow the progression of this disease.

3.6. Extracellular matrix remodelling

Parallel to neovessels formation, diseased aortic valves are places of intense remodeling of the extracellular matrix favoring disease progression. Several matrix metalloproteases (MMPs), a family of zinc-dependent endopeptidases that degrade extracellular matrix [73] are increasingly expressed in stenotic aortic valves with respect to controls. In particular, an increase in MMP-1 [46,49,74], that co-localizes in aortic valves with TNF-α has been observed, which suggests an important link between matrix remodeling and inflammation [49]. In addition, other MMPs are increased in this pathological condition, as MMP-3 [74,75], MMP-9 [74—77]. Concerning MMP-2, even if some studies have provided evidence for a potential involvement of this MMP in the development of aortic valve disease, others have not confirmed this finding [74,76,78—80]. The behavior of tissue inhibitors of MMP-1 and MMP-2 (TIMPs) is even more controversial: some studies failed to show TIMPs parallel increases [49] or have shown minor increases [77], whereas others have documented sensible increases of these
of atherosclerosis the same fortune, a mix of success and failure, has been a subject of debate over the years, sharing with the infective hypothesis that infectious agents may play a role in the development of calcific aortic valve disease. However, the exact nature and role of these agents in calcific aortic disease are still not fully understood. Studies have suggested that pathogens such as Chlamydia pneumoniae or mast cells may contribute to the calcification process.

3.7. Oxidative stress

It has been documented that, in stenotic aortic valves, oxidative stress is increased, as shown by higher levels of superoxide and hydrogen peroxide compared with controls. This increased oxidative stress is associated with an increase in reactive oxygen species (ROS) and an elevation of pro-inflammatory mediators. These findings suggest that oxidative stress may play a role in the pathogenesis of calcific aortic disease. The increased oxidative stress is likely due to the activation of NADPH oxidase and other oxidant enzymes, which contribute to endothelial damage and the release of proteolytic substances that damage endothelial cells and contribute to subendothelial matrix deposition.

3.8. Infection

The hypothesis that an infective agent, Chlamydia pneumoniae, was related to development and progression of the aortic valve stenosis, has been pursued by several authors over the years, sharing with the infective hypothesis of atherosclerosis the same fortune, a mix of success and failures. Both for aortic sclerosis and for aortic valve stenosis, studies addressing the potential association between chlamydia and calcific aortic disease have not provided definitive answers. Regarding the possible role of other infectious agents, Bratos-Perez et al. have recently shown that a significant percentage of severely stenotic aortic valves collected at surgery (48/75, 64%), once cultured in appropriate media, show the presence of growing self-replicating calcifying nanoparticles, also called nanobacteria, that potentially represent new pathogens under scrutiny, whose presence has already been found in carotid disease, abdominal aorta aneurysms, and in calcific human vessels or valves. Nevertheless, further evidence is needed for a clear demonstration of the role of these agents in calcific aortic disease.

4. Bone metabolism

The role of calcium metabolism in calcific aortic stenosis has always been considered definite as for several decades calcific aortic stenosis has been associated with diseases characterized by perturbations in calcium metabolism such as chronic renal failure and hemodialysis. This role has then been confirmed by several papers demonstrating a possible direct association with ionized calcium and an inverse one with total serum calcium. In addition, aortic stenosis has been related with higher serum parathyroid hormone and lower vitamin D levels in male patients affected by aortic stenosis and coronary disease. These data, together with evidence concerning a higher frequency of the B allele of vitamin D in patients with aortic stenosis (see Section 2), confirm the importance of systemic calcium metabolism, especially in terms of increased calcium mobilization, in the progression of this disease.

In addition, even if the concept of calcification has always been related to nonrheumatic aortic stenosis (which has frequently been called calcific) the process underlying calcification and, eventually, bone remodeling has been attributed for nearly a century to a passive degenerative process. Only recently, several studies have documented that calcium deposition and bone formation in stenotic aortic valves is an actively regulated process and that the aortic valve specimens collected during aortic valve replacement show heterotopic ossification, active bone remodeling and mature lamellar bone. In addition, in these valves there are increased levels of several osteoblast markers such as osteopontin, osteocalcin, osteoprotegerin, bone sialoprotein, and of the osteoblast-specific transcription factor Cbfal. Moreover, the endochondral process of bone formation leading to aortic valve calcification seems to share several features with mature bone, and is strictly associated with inflammation and neoangiogenesis; besides, the pathway Lrp5/Wnt, osteoblast differentiation signaling markers that play a primary role in bone development, and the osteoprotegerin/RANKL (the RANK receptor) axis are also involved in aortic valve calcification. RANKL, a member of TNF-κ superfamily, is a transmembrane protein expressed on osteoblasts surface, stromal cells, T cells and endothelial cells, that interacts with the transmembrane protein RANK on osteoclasts precursors or on mature osteoclasts promoting their differentiation via NF-κB. This interaction can be
effectively blocked by circulating osteoprotegerin that is able to stop osteoclasts differentiation [109,110]. Both in aortic sclerosis [54] and in aortic stenosis [53,54,111] the relative concentrations of the components of this axis are sensitively changed with respect to controls, with increases in RANK and RANKL and a decrease in osteoprotegerin, a pattern promoting aortic valve calcification. Current data suggest that the process of aortic valve calcification is thus a multi-factorial event where several pathways converge to enhance disease progression, a complex mechanism that might request a multi-target approach for its modulation. The recent demonstration that adrenergic and purinergic pathways can also contribute to this process, confirms and expands the concept of the need of multiple target modulation. Osman et al. have in fact shown that β-adrenergic receptors, especially β2 ones, are upregulated in stenotic valves, mainly in peri-calcific areas [111], and the prolonged incubation of cultured interstitial aortic valve cells with a stable analogue of the purinergic receptor P2Y (ATP-γ-S) causes their differentiation into osteoblasts [112], further supporting a multi-effector and multi-cause process.

5. Fluid mechanics

In addition to the role of genomics, atherosclerosis and bone metabolism, fluid mechanic perturbations that progressively occur at the site of aortic stenosis may themselves contribute to the progression of the disease amplifying the biological changes that underlie the evolution of aortic stenosis. As blood velocity increases through a stenotic aortic valve, local endothelial shear stress changes and, ultimately, turbulent flow occur. This can activate endothelial cells, tipping the balance of endothelial-derived factors to disrupt barrier function, and enhance coagulation, leukocyte adhesion, and smooth muscle cell proliferation [113]. Even if these responses likely exist as protective mechanisms, these changes may render these responses excessive, resulting in damaged tissue, impaired organ function, and an abnormal fibroproliferative response. Besides the previously described effects on hemostasis via von Willebrand factor perturbations, local shear stress changes can affect several different pathways related with the progression of aortic stenosis; among them cell cycle, nitric oxide and prostacyclin release, oxidative stress, lipoprotein uptake, synthesis and permeability, inflammation, vascular muscle cell migration, differentiation and proliferation, and neoangiogenesis [114—117]. Finally, it is interesting to note that different shear stress patterns can determine the endothelial and smooth muscle cell phenotype towards activation or quiescence [118,119], further supporting the role of fluid mechanics in aortic stenosis.

6. Pharmacologic modulation of calcific aortic stenosis progression: basic mechanisms and clinical results

From the previous sections it is clear that several mechanisms involved in atherosclerosis are also possibly involved in the development and progression of calcific aortic stenosis; the class of drugs more widely investigated, on an experimental ground in a clinical practice, are, as a consequence, the HMG-CoA reductase inhibitors, named statins. It is well known that statins exert significant anti-inflammatory effects and that they also preserve endothelial function in the general population [120,121], and this concept might be translated into potential beneficial effects for patients affected by aortic valve disease [1,19,122], although a definitive demonstration of the ‘aortic protection’ is unfortunately still lacking. Atorvastatin reduces the progression of aortic valve calcification in rabbits fed with an atherogenic diet via Lrp5 pathway [123] and endothelial nitric oxide synthase [124] modulation; in vitro studies have also shown that atorvastatin reduces the activity of alkaline phosphatase, a marker of osteoblast activity, in cultured interstitial aortic cells previously incubated in osteogenic media obtained with the addition of compounds with purinergic activity [112]. Other studies have assessed the role of statin treatment on different cell populations of the aortic valve showing that, in animal cell cultures, these drugs exert their maximal effect by limiting the formation of calcific nodules and alkaline phosphatase activity of aortic myofibroblasts via downregulation of HMG-CoA reductase activity; on the other hand, in osteoblasts, statins favor alkaline phosphatase activity, and they also promote the differentiation in osteoblasts of other cell populations such as bone marrow stromal cells [125]. This effect, called ‘statin paradox’, suggests the hypothesis that timing of statins administration may be critical in reducing disease progression, as the prevailing cell populations during the various phases of the development of aortic stenosis may differ, as well as the response to drug treatment. Other potentially beneficial biological effects of statins include the attenuation in the expression of pro-inflammatory mediators, although this modulation is incomplete. Indeed, statins reduce the expression of the inflammatory markers eosetin-3, and of a monokine induced by interferon-gamma, but not of TGF-β and of vascular adhesion protein-1 [47]. Another proof of paradoxical biological effects of statins is the demonstration that these drugs reduce the expression of regulators of G protein-mediated signaling (RGS) proteins RGS2, RGS3 and RGS4 in stenotic aortic valves leading to an increase in the activation of extracellular-regulated kinases [126] that in turn stimulate further proliferation of myofibroblasts; this suggest that, even in this setting, not all the effects of statins are potentially beneficial for prevention of stenotic aortic disease progression. This is not totally unexpected, due to the complex pleiotropic activities of statins on the overall isoprenoid pathway. Indeed, inhibition of the HMG-CoA reductase enzyme by statins, results in the inhibition not only of cholesterol biosynthesis, but it influences the generation of a variety of isoprenoid compounds, important in cell cycle, inflammation and osteogenesis, as well.

Concerning clinical studies, both retrospective and prospective studies have addressed the question whether statin therapy can reduce the progression of this disease, reaching controversial results, as the majority of retrospective studies document a protective effect of statins on aortic valve stenosis evolution, whereas the majority of prospective and all prospective and randomized trials do not. The prospective randomized SALTIRE has enrolled patients
affected by aortic stenosis with total cholesterol levels ≥150 mg/dl and with a jet velocity ≥2.5 m/s; overall, patients average jet velocity was 3.42 m/s, with a peak gradient of 48 mmHg, and an aortic valve area of 1.03 cm². The results of SALTIRE show that a high-dose (80 mg/die) of atorvastatin therapy fails to show any effect, at a median follow-up of 25 months, on the primary end-points of the study, assessing aortic stenosis progression. The echocardiographic annual change in jet velocity was 0.199 m/s in atorvastatin-treated patients, and 0.203 m/s in placebo-treated (p = 0.95), whereas the valve calcium score increase, assessed with helical computed tomography, was 22.3% and 21.7% in statin and placebo group, respectively (p = 0.93) [127]. Unlike SALTIRE trial, the RAAVE trial, a prospective open-label study assessing the effect of rosuvastatin treatment on aortic stenosis over 18 months, has documented significant improvements in aortic stenosis progression in statin-treated patients. With respect to SALTIRE, patients enrolled in RAAVE had higher aortic valve areas (1.23 cm²) but similar jet velocities (3.63 m/s) at baseline; in this latter study, treatment was not assigned by randomization, but patients presenting with abnormally high LDL cholesterol levels (>130 mg/dl) received rosuvastatin, whereas patients with lower LDL levels received no statin. RAAVE has shown that aortic valve area decreased annually in both groups, but statin-treated patients had half the progression with respect to untreated patients (decrease in aortic valve area 0.05 vs 0.1 cm² per year, p = 0.04) [128].

Recently, results of the prospective randomized study SEAS, the biggest one up to date in this field, have been published; in this study 1873 patients with LDL values <236 mg/dl, with an average aortic valve area of 1.28 cm² and an average jet velocity of 3.1 m/s, were enrolled; these patients have been randomized to be treated for a minimum of 4 years with placebo or ezetimibe/simvastatin, for a median follow-up of 52.2 months. In SEAS, ezetimibe/simvastatin was no better than placebo in reducing the primary composite end-point of aortic valve-related and cardiovascular events (defined as death from cardiovascular causes, aortic valve replacement, congestive heart failure as a result of progression of aortic valve stenosis, nonfatal myocardial infarction, hospitalization for unstable angina, coronary artery bypass grafting, percutaneous coronary intervention, or non-hemorrhagic stroke), occurring similarly in treated patients (35.3%) and in controls (38.2%, p = 0.59). In addition, the secondary end-point of aortic valve-related events (defined as aortic valve replacement surgery, congestive heart failure due to aortic stenosis, or death from cardiovascular causes) did not differ between groups, occurring in 32.6% and 35.1% of cases and of controls, respectively (p = 0.73). On the other hand, this treatment was significantly more effective than placebo in reducing the risk of ischemic events, a secondary composite end-point driven primarily by reductions in coronary artery bypass graft surgery; this occurred in 15.7% and 20.1% of cases and of controls, respectively (p = 0.02) [129]. In addition, this study has raised some concerns over the significantly increased risk of cancer of treated patients being doubled in patients who received ezetimibe/simvastatin. The new cancers, however, were not specific to one site, and an interim analysis of ongoing ezetimibe/simvastatin studies SHARP and IMPROVE-IT, plus the analysis from SEAS, revealed no increased risk [130].

Finally, the results of another randomized trial, ASTRONOMER, are expected soon; this study will address the effect of rosuvastatin (40 mg/day) treatment on aortic valve stenosis progression in 272 patients with an average jet velocity of 3.2 m/s, an aortic valve area of 1.2 cm², a mean transvalvular gradient of 22 mmHg, half of them having bicuspid aortic valve, for a 3- to 5-year follow-up period [131]. In addition, other prospective randomized studies concerning statins and aortic valve disease are currently running, and the results of a search of these trials on the site www.clinicaltrials.gov, performed on 26/08/2008), is reported in Table 1.

As stated before, evidence from retrospective nonrandomized studies is in contrast with what has been previously shown by randomized ones. Although publication bias in this case cannot totally be excluded, data from these studies consistently show that statin therapy reduces the progression of aortic valve disease, whatever the degree of stenosis was documented at the beginning of follow-up, and this result has been documented both with echocardiography [132–135] or with CT scan for detection of calcium content of the aortic valve [40,136,137].

Based on these data, we can infer that statins are a class of drugs with potentially beneficial effect on the reduction of valve disease progression, but this is not demonstrated yet; it is also possible, in our opinion, that timing is the most critical factor for the optimal efficacy of this class of drugs, and that their maximal effects are obtainable at earlier stages of the disease such as aortic sclerosis. In support of this hypothesis, a recent retrospective study of Antonini-Canterin et al. analyzing 1046 patients over 19 years has shown that, at an average follow-up of 5.6 ± 3.2 years, statins can reduce aortic stenosis progression only in the milder degrees of the disease, such as in case of aortic sclerosis (jet velocity ≥1.5 and <2.0 m/s) or in case of moderate aortic stenosis (jet velocity ≥2 and <3 m/s). When aortic stenosis was more severe (jet velocity ≥3 and <4 m/s), the effect of statins was null [138]. If this will be confirmed by appropriate studies, it may strongly impact prevention programs of health systems that will have to consider screen for and to treat aortic sclerosis. It should be noted, however, that all the randomized trials done to date or still running might have unfortunately missed the patient population where statins could be more effective, as all studies have enrolled patients with jet velocities almost always >3.0 m/s, and therefore might fail to provide evidence of the potential benefits of statins in the relatively early phases of abortive valve disease, namely during the phase of aortic sclerosis. Finally, it is well known that the progression of sclerosis and stenosis and especially of sclerosis toward stenosis is substantially unpredictable in the individual patient, being in some cases very slow, in others quite accelerated, and that different patterns of aortic valve disease progression (linear and non-linear) are possible [139]; this, together with evidence concerning the potential beneficial effects of statins in aortic valve sclerosis and not in aortic valve stenosis, suggests that the assumption that valve sclerosis is always and invariably the precursor of valve stenosis may not be completely correct.

Converting enzyme inhibitors are another class of drugs with potentially beneficial effects on aortic stenosis
progression as the presence of converting enzyme has been shown both in sclerotic [18,32] and stenotic aortic valves [32,140], as well as kinase [140]; both these enzymes are involved in angiotensin II production, a molecule with pro-inflammatory and profibrotic activity; but clinical data, although scarce, actually do not show any effect of these drugs on this disease [135].

Moreover, preliminary evidence from animal models suggest that olmesartan, an angiotensin type 1 receptor antagonist, reduces atherosclerotic changes in aortic valves by preserving endothelial cells integrity and inhibiting transdifferentiation into myofibroblasts or into osteoblasts in valve leaflets [141], but no clinical data about this class of drugs is still actually available.

Finally, another potential therapeutic target to reduce aortic stenosis progression is smoke cessation. It has been shown that nicotine and acetaldehyde, both smoke components, induce TGF-β1 expression in cultured fibroblasts, and nicotine can also activate mast cells; both these mechanisms can ultimately lead to an increased collagen/elastin ratio in valve tissue [81]; this finding suggests a potential role also for smoke cessation in the prevention of calcific aortic disease.

7. Conclusion

This review underlines the concept that the progression of calcific aortic stenosis is a multi-factorial process resembling the atherosclerotic process, with nevertheless some important differences in the final results achieved, as in atherosclerosis the final result is plaque development and plaque instability whereas calcific aortic disease ends up as severe calcification of the aortic valve. This suggests that calcific aortic stenosis is not a single disease process but it seems to be more likely a common macroscopic anatomic equivalent of a series of partly related processes that ultimately lead to severe calcification of the valve.

The multitude of the mechanisms potentially involved in aortic disease progression, together with the available clinical evidence strongly suggest that drug therapy aimed at reducing this progression will necessarily have to be multifactorial and will have to address the earliest stages of the disease, as it is now clear that drug therapy administered in more advanced stages of the disease may be ineffective or, at best, much less effective. The time has come to integrate all the possible mechanisms affecting the development and progression of aortic stenosis into a unique effort to discover innovative therapies that may lead to lower evolution over time.

References


